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Familial X-linked Mental Retardation and Isolated Growth Hormone Deficiency: Clinical and Molecular Findings

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We report on several members of a family with varying degrees of X-linked mental retardation (XLMR), isolated growth hormone deficiency (IGHD), and infantile behaviour but without other consistent phenotypic abnormalities. Male patients continued to grow until well into their twenties and reached a height ranging from 135 to 159 cm. Except one, all female carriers were mentally normal; their adult height ranged from 159 to 168 cm. By linkage studies we have assigned the underlying genetic defect to the Xq24-q27.3 region, with a maximum lod score of $Z = 3.26$ at $\theta = 0.0$ for the DXS294 locus. The XLMR-IGHD phenotype in these patients may be due to pleiotropic effects of a single gene or it may represent a contiguous gene syndrome.

KEY WORDS: **X-linked mental retardation, isolated growth hormone deficiency, gene localization**

INTRODUCTION

X-linked mental retardation (XLMR) is an important cause of mental handicap. Opitz [1986] calculated the prevalence of all XLMR at 1/296 for both sexes. Neri et al. [1994] listed 127 conditions with XLMR, including various nonspecific forms. In contrast, X-linked isolated growth hormone deficiency (IGHD) is very rare [Phillips and Cogan, 1994; Perez Jurado and Argente, 1994]. IGHD has been reported in combination with agammaglobulinemia at Xq21.3-q22, the site of the X-linked agammaglobulinemia (XLA) gene [Duriez et al., 1994]. Growth hormone deficiency has also been described in a boy with a dup(X)(q13.3-q21.2) [Yokoyama et al., 1992], and Ogata et al. [1992] have hypothesized on the localization of growth gene(s) in the pseudoautosomal region. Moreover, growth hormone deficiency is one manifestation in patients with X-linked recessive panhypopituitarism, a rare but well-defined genetic condition [Phelan et al., 1971; Schimke et al., 1971; Zipf et al., 1977].

While there are forms of XLMR that are associated with short stature [Neri et al., 1994], the combination of XLMR with IGHD has not been described yet. Here we report on a family in which mental retardation and IGHD co-segregate as an X-linked trait. Linkage studies have assigned the underlying gene(s) to the distal half of Xq thereby indicating that we are dealing with a "novel" disorder.

MATERIALS AND METHODS

Clinical Report and Family History

A four generation family included eight male patients with variable mental retardation and short stature (Fig 1). The propositus (III-9) was examined in 1976 by an endocrinologist at the age of 28 years when the diagnosis of IGHD was made. He was born at term, after an uneventful pregnancy and delivery. Motor development was normal. He attended a special school for children with learning difficulties and thereafter worked in a sheltered environment. At the age of about 13 years he weighed 32 kg at a height of 127 cm. There were no health complaints. He shaved once or twice a week, and had regular erections and ejaculations. Remarkably he had continued to grow into his twenties. Age at pubarche is unknown. On examination height was 155 cm. Besides mild gynaecomastia, no other abnormality was seen; in particular he had normal external genitalia, with a testicular volume of 20 ml. Laboratory investigations gave normal results for cortisol, thyroxine, T3, testosterone, LH, and FSH. GH concentrations before and after insulin induced hypoglycaemia were all below 1 mU/L. Radiographs of the skull showed a small sella turcica. He had an adult bone age.

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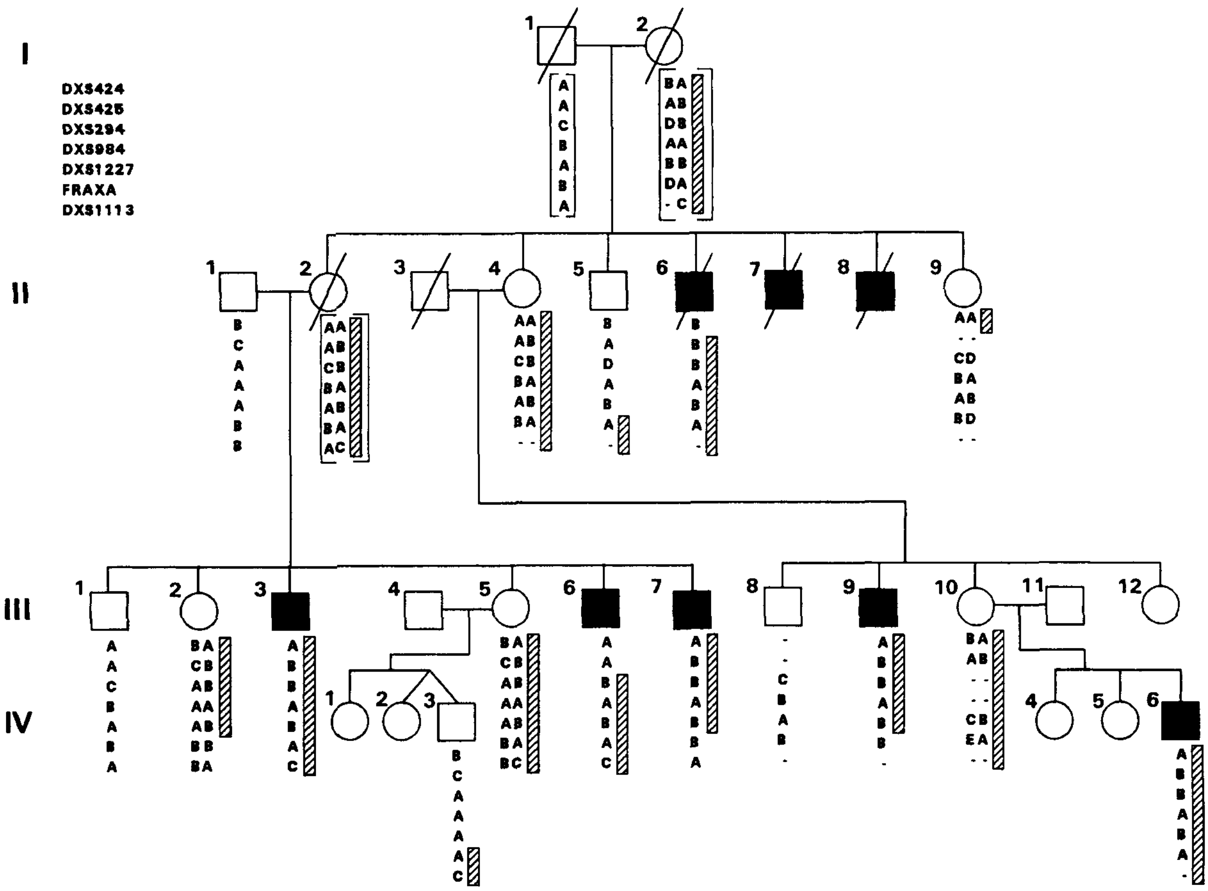

Fig. 1. Pedigree of the family with X-linked mental retardation and isolated growth hormone deficiency. Haplotypes are shown, which have been constructed from informative markers in the region Xq24-q28. The co-segregating haplotype has been marked by a cross-hatched bar.

When seen again at the age of 45 years (Fig. 2), he showed an infantile behaviour. Results of physical and laboratory examination are given in Tables I and II.

II-6 died for unknown reasons at the age of 18 years. He was mildly mentally retarded and attained a height of 143 cm.

II-7 died at the age of 72 years because of leukemia. Reportedly he was mentally dull and had a height of 158 cm.

II-8 died at the age of 51 years because of food aspiration. He had been institutionalized because of mental retardation. His height was 135 cm. At autopsy the sella turcica and the pituitary gland appeared very small; the latter weighed 170 mg, while the normal mean adult weight is about 600 mg [Kovics and Horvath, 1986].

Patients III-3, III-6, III-7, and IV-6 were all born at term after an uneventful pregnancy and delivery.

III-3 was severely growth retarded and attended a special school for children with learning difficulties. At the age of 20 years he had a height age of 10 years and a bone age of 12 years. He continued to grow into his twenties. Psychometric testing (WAIS) at the age of 47 years demonstrated a total IQ of 81 (verbal: 89, performance: 74). When seen at the age of 50 years (Fig. 3), he used antiepileptic therapy, though he had not had convulsions for a long time. Otherwise he was healthy. His behaviour was infantile. He lived and worked in a sheltered environment. Results of physical and laboratory examination are given in Tables I and II.

III-6 was also short statured and attended a special school for chidden with learning difficulties. At the age of 16 years he had a height age of 8 1/2 years and a bone age of 10 years. He reached his final height in his twenties. When seen at the age of 45 years (Fig. 4), he lived and worked in a sheltered environment and did not have any health problems. He showed an infantile behaviour. Results of physical and laboratory examinations are given in Tables I and II.

III-7 had, as the first in his family, postaxial polydactyly of both hands. Postnatally he was operated for a possible duodenal stenosis. He also was short statured. He was institutionalized for his mental retardation. At the age of 11 years he had a height age of 7 years and a bone age of 5 years. At age 16, he weighed 33 kg at a height of only 130 cm, but he, too, continued to grow into his twenties. He was seen at the age of 39 years (Fig. 5). There were no particular health prob-

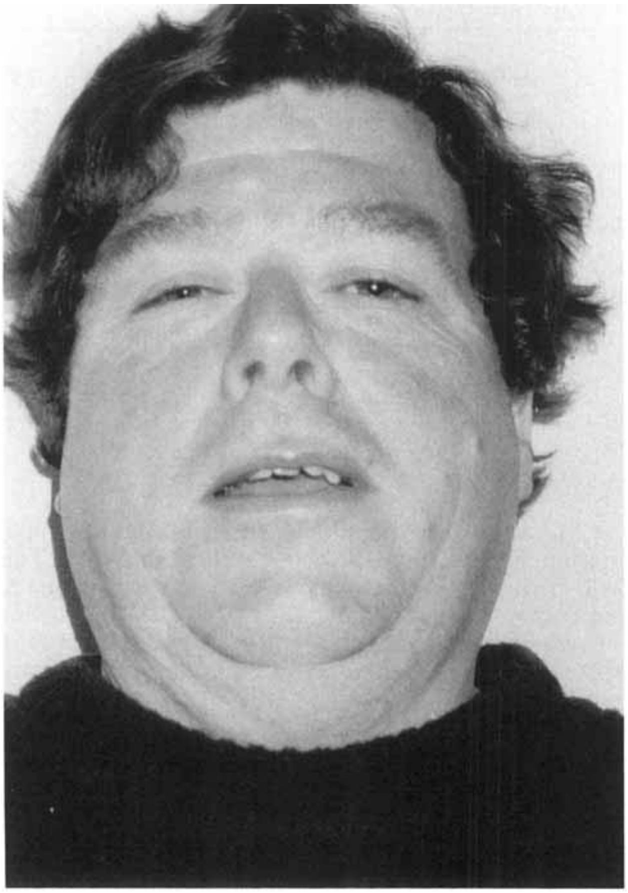

Fig. 2. Patient III-9 at the age of 45 years.

lems. Results of physical and laboratory examinations are given in Tables I and II.

IV-6 had a birth weight of 4,500 g. Motor milestones were reached within normal limits. Gradually he became short statured with truncal obesity and a puffy face, as seen in hypothalamic disorders. At the age of 4 years, he weighed 20 kg and his height was 100 cm. He attended a special school for children with learning difficulties. In course of time many investigations were performed with normal results for thyroid function, cortisol, LH/FSH/TSH/prolactine after LH-RH, and TRH stimulation. L-DOPA and arginine GH stimulation tests gave maximal GH levels of 6 mU/L, thereby providing clear evidence for a total GH deficiency. Cerebral CT-scan was normal and radiographs of the skull showed a small sella turcica. At the age of 9 years and 8 months his bone age was 7.9 years. At the age of 10 years GH replacement therapy was started. Because of secondary hypothyroidism during GH therapy, thyroxine was supplemented at the age of about 14 years. At the age of 15 years he had not yet entered puberty. He showed an infantile behaviour. Results of physical and laboratory examinations just before the start of GH treatment are given in Tables I and II.

Figure 6 shows height growth curves of patients II-7, II-8, III-9 and IV-6.

Obligate carriers (I-2, II-2, II-4, and III-10) and carriers identified by linkage analysis with flanking markers (III-2 and III-5) had heights ranging from 159 to 168 cm, i.e., at or above the 10th centile; they were all mentally normal with the exception of III-2 who attended a special school for children with learning difficulties and remained unmarried.

Linkage Studies

Blood sampling was performed and genomic DNA was extracted from all available relatives [Miller et al., 1988]. Genotyping with microsatellite markers (Table III) involved PCR amplification of 50 ng genomic DNA in 15 μl 1× Supertaq^R buffer [10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% (w/v) gelatin] in the presence of ^{32}P-dCTP with 0.06 U Supertaq^R (HT Biotechnology, Ltd, England). Amplification was achieved by 35 cycles of 1 min at 94°C, 2 min at 55°C and 3 min at 72°C with the appropriate primers (Genome Database; Isogen Bioscience, The Netherlands). Subsequently, the labeled amplification products were separated by electrophoresis on 6.6% denaturing polyacrylamide gels. To visualize the allelic

TABLE I. Summary of Selected Clinical Findings (Centiles)*

	III-3	III-6	III-7	III-9	IV-6
Age (yr)	50	45	39	45	9yr, 5mo
Height (cm)	144(<3)	159(<3)	156(<3)	155(<3)	125.8(<3)
Span (cm)	145	162	155	157	nd
OFC (cm)	52.4(<3)	54(10–50)	52.6(<3)	53.5(3–10)	52(10–50)
Ears (mm)	74(75–97)	65(75)	65(75)	69(75–97)	nd
Testes (ml)	20(50)	20(50)	15(10–50)	20(50)	pp
Gothic palate	+	+	+	−	−
Dental crowding	+	+	+	−	−
Gynaecomastia	+	+	+	+	−
Normal external genitalia	+	+	+	+	+
Clinodactyly	+	+	+	−	−
Mild scoliosis	+	−	+	−	−

*OFC, Occipito-frontal circumference; +, present; −, not present; nd, not done; pp, prepubertal; yr, year; mo, months.

TABLE II. Summary of Laboratory Investigations*

	Normal values	III-3	III-6	III-7	III-9	IV-6
Somatomedin C nmol/L	12–48[a]	3.5	10.3	6.7	9.7	8.2
Testosterone nmol/L	10–25	36	13	27	18.2	<2[b]
Estradiol nmol/L	0.03–0.11	0.08	0.06	0.08	0.03	nd
Prolactin mE/L	100–700	nd	310	500	140	310
T4 nmol/L	54–154	67	91	93	72	90
Free T4 pmol/L	9–17	nd	10.3	11	nd	12.1
TSH mE/L	0.4–4.0	3.5	1.94	1.89	nd	3.8
Cortisol μmol/L	morning 0.19–0.55	nd	nd	nd	0.60	0.22

*nd, not done.
[a]In all 5 patients : <3rd centile for age.
[b]Normal for his age (prepubertal).

bands, gels were dried and exposed overnight to Kodak X-OMAT S film. Lod score calculations were performed with the Mlink option of the program LINKAGE version 5.10 [Lathrop et al., 1985]. Complete penetrance was assumed and the disease gene frequency was estimated at 0.00001. Allele frequencies for each of the markers were assumed as reported in GDB.

RESULTS

Clinical examination demonstrated facial anomalies in some but not in all of the patients (Table I and Figs. 2–5). In adult patients, gynaecomastia was seen. All patients were short and mentally retarded, ranging from a severe impairment necessitating admission to an institute for the mentally handicapped, to dull mentality.

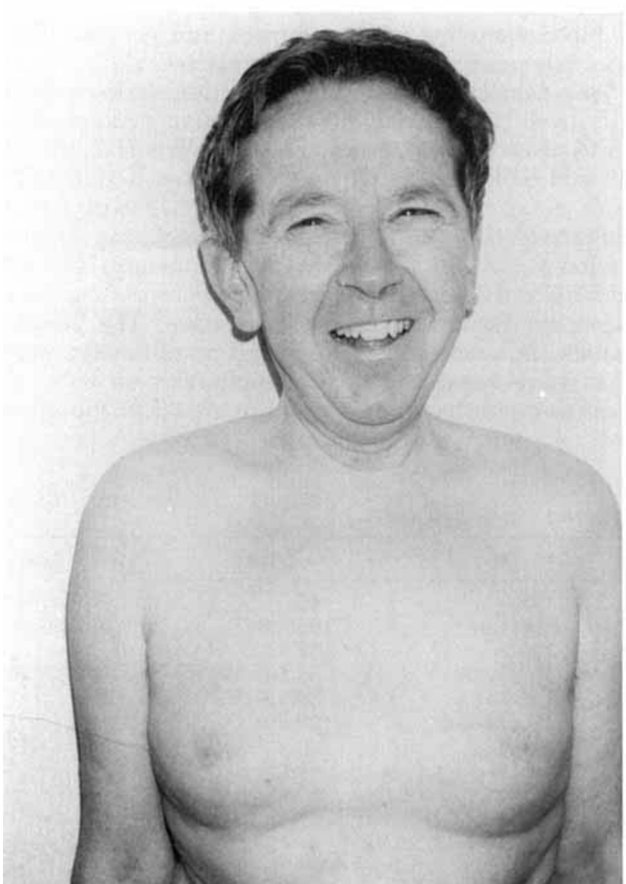

Fig. 3. Patient III-3 at the age of 50 years.

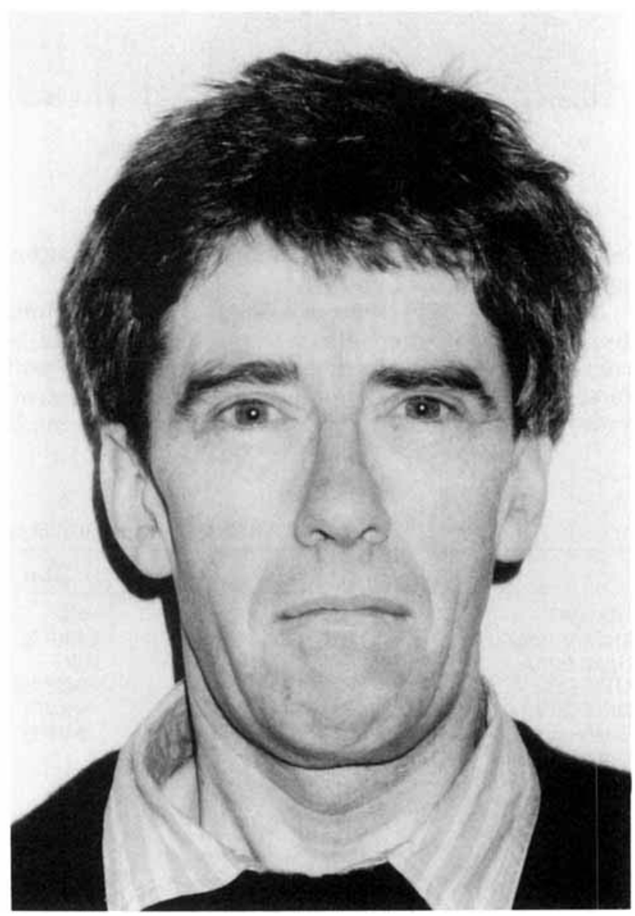

Fig. 4. Patient III-6 at the age of 45 years.

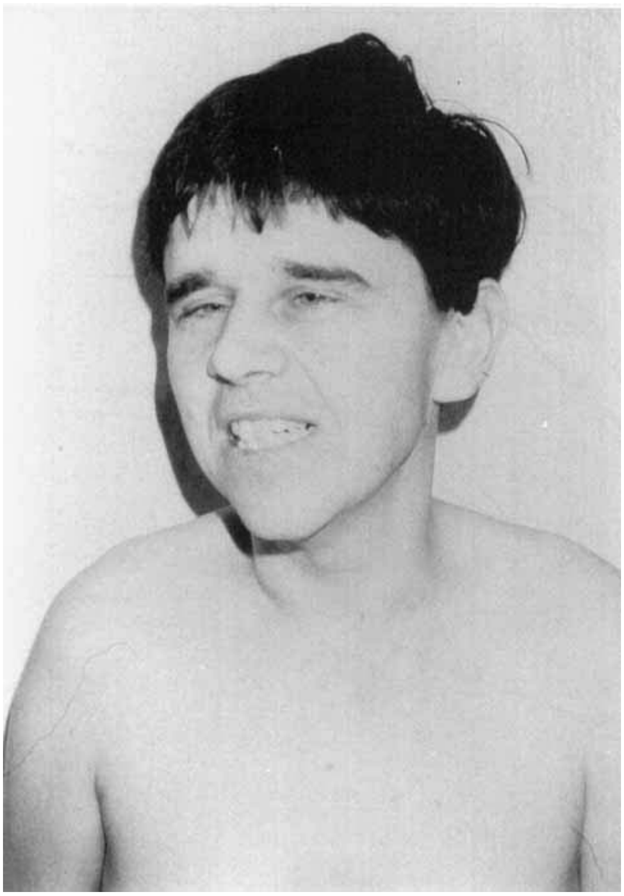

Fig. 5. Patient III-7 at the age of 39 years.

On the average, untreated patients reached their final height, ranging from 135 to 159 cm, at the age of 24 to 25 years (Fig. 6). In all examined patients, behaviour was considered infantile, and laboratory investigations demonstrated total IGHD (Table II). In several patients high resolution G- and Q-banded chromosomes were normal and the FRAXA CCG repeat size was normal.

Linkage analysis was performed with 28 highly polymorphic microsatellite markers distributed more or less evenly along the entire X chromosome. In Table III, the markers are listed in the Xpter→Xqter order based on the recent map of Fain et al. [1995]. Significant lod scores were only obtained with markers DXS294, DXS984 and DXS1227 indicating that the responsible genetic defect is located in the distal part of Xq. The maximum lod score was $Z = 3.26$ at $\theta = 0.0$ for marker DXS294. To locate the genetic defect more accurately, haplotypes were constructed with markers from the relevant region (Fig. 1). In this way, DXS425 and FRAXA were defined as the closest proximal and distal markers, respectively, spanning a distance of 40 cM and thereby excluding involvement of the FRAXE gene. Thus, our results demonstrate that the genetic defect underlying mental retardation and growth hormone deficiency in this family is located in Xq24-q27.3.

DISCUSSION

X-linked mental retardation with IGHD has, to our knowledge, not yet been described before.

Many XLMR genes have been assigned regionally [Neri et al., 1994]. Those assigned to Xq24-q27.3 concern well-described syndromes, metabolic and neuromuscular disorders, which are clinically clearly different from what is seen our patients [Neri et al., 1994]. Of the nonspecific forms, MRX6 maps to Xq26 and clinically it combines mental retardation with short stature, "coarse" face with other facial anomalies and stubby hands [Kondo et al., 1991]. It is not stated whether the small body size was due to growth hormone deficiency or to other causes. X-linked recessive panhypopituitarism is excluded in our family because clinical and endocrinological examinations gave no evidence for general hypothalamic/hypophyseal dysfunction other than lack of growth hormone. Likewise, the small sella turcica as well as the size reduction of the pituitary gland seen in one patient on autopsy is compatible with an isolated deficiency of growth hormone producing cells, which account for most cells of the adenohypophysis [Kovics and Horvath, 1986]. IGHD was been described previously in combination with XLA. XLA or Bruton type agammaglobulinemia is due to mutations in the BTK gene, which is located in the Xq21.3-q22 interval [Duriez et al., 1994]. Our patients did not have an apparent increased susceptibility for infections, but, more importantly, the localization of the involved gene(s) in this family is in a different region of the X chromosome. Yokoyama et al. [1992] and Ogata et al. [1992] provide evidence for the presence of genes involved in GH regulation to Xq13.3-q21.2 and the pseudoautosomal region on the distal Xp, respectively. Our study suggests that the Xq24-q27.3 interval contains gene(s) involved in mental development and in GH regulation. The gene(s) underlying X-linked IGHD has (have) not been mapped yet. Therefore at least formally, it is possible that in the family reported here, such a gene is involved, as well as a hitherto undescribed gene for XLMR. For that reason we cannot rule out the possibility that the combination of XLMR and IGHD segregating in this family as a Mendelian trait is a contiguous gene syndrome resulting from a microdeletion spanning two closely linked genes in the distal long arm of the X-chromosome. In the absence of independent evidence for the existence of separate genes for XLMR and IGHD in the relevant Xq24-q27.3 interval, however, the alternative, i.e., that the XLMR-IGHD phenotype is due to pleiotropic effects of a single gene defect, may be more plausible. Indeed, one form of XLMR with short stature has been mapped to the same interval [Kondo et al., 1991]. Unfortunately, the wide linkage interval does not yet allow reliable carrier detection and prenatal diagnosis.

Only the youngest patient had GH replacement therapy, with apparent success. All his untreated affected relatives continued to grow into their twenties, which can at least partly be explained by the severely retarded bone age, and entered puberty without treat-

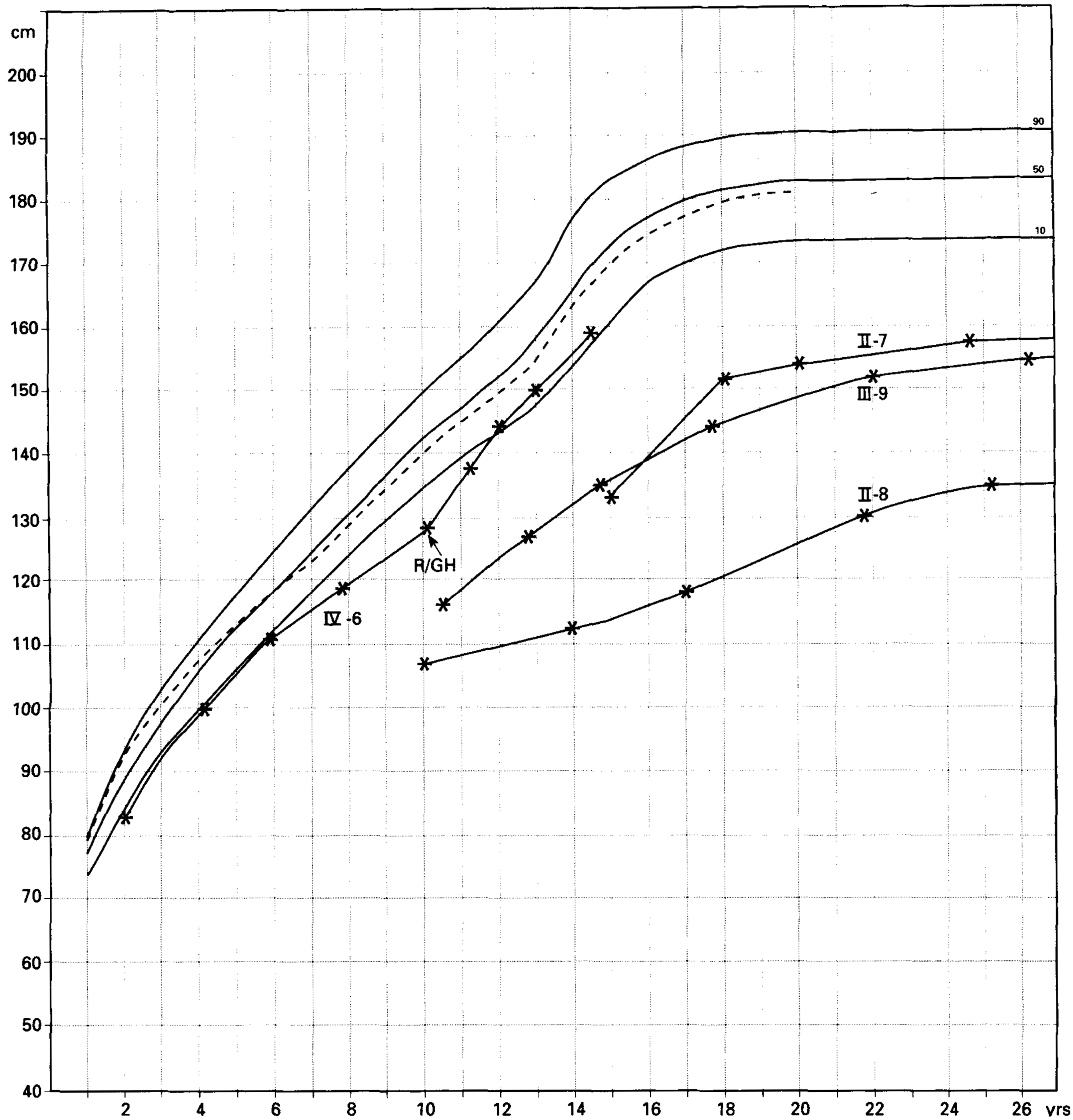

Fig. 6. Height growth curves of patients II-7, II-8, III-9, and IV-6.

ment. Their final height ranged from 135 to 159 cm. A similar range was found by Wit et al. [1996] when pooling literature and personal data on final height of untreated patients with severe IGHD and spontaneous puberty. Untreated adult GH deficiency exhibits a whole array of metabolic effects ranging from reduced bone mineral content to increased cardiovascular mortality. Impaired psychological well-being has been reported as well. GH replacement therapy can totally or partially alleviate these clinical consequences and is therefore advisable in adult GH deficient patients [Jorgensen et al., 1994; de Boer et al., 1995]. In adequately treated IGHD-patients puberty will occur at normal age and final height may approach target height [Wit et al., 1996].

In conclusion we report on a family in which a hitherto unreported combination of MR and IGHD cosegregates in an X-linked fashion. Linkage studies suggest a location of the responsible gene(s) in the Xq24-q27.3 interval. The regional assignment of the genetic defect in this family is a first step towards the elucidation of the underlying gene(s) and the causative mutation(s).

Table III. Results of Two-Point Linkage Analysis*

Marker	LOD scores (θ) 0.0	0.05	0.1	0.2	0.3	0.4
DXS1060	−∞	−3.22	−1.88	−0.72	−0.21	−0.00
GHGXg	−∞	−0.08	0.11	0.19	0.15	0.08
DXS443	−0.20	−0.17	−0.15	−1.10	−0.06	−0.03
DXS451	−∞	−0.54	−0.07	0.25	0.29	0.19
DMD	−∞	−1.48	−0.98	−0.55	−0.33	−0.16
DXS538	−∞	−1.08	−0.59	−0.22	−0.09	−0.03
DXS7	−0.20	−0.17	−0.15	−0.10	−0.06	−0.03
MAO A	−∞	−0.92	−0.62	−0.31	−0.15	−0.05
DXS1003	−∞	−0.26	0.21	0.51	0.49	0.31
ALAS2	−∞	−0.74	−0.46	−0.21	−0.08	−0.02
DXS453	−∞	−0.64	−0.37	−0.13	−0.03	0.00
DXS559	−∞	−0.74	−0.46	−0.21	−0.08	−0.02
DXS3	0.22	0.21	0.19	0.15	0.11	0.06
DXS1231	−∞	−5.06	−3.34	−1.73	−0.87	−0.34
DXS178	−∞	−4.20	−2.54	−1.06	−0.37	−0.06
COL4A5	−∞	−0.88	−0.50	−0.37	−0.44	−0.32
DXS425	−∞	0.99	1.04	0.82	0.46	0.12
DXS424	−∞	−0.08	0.12	0.22	0.18	0.10
HPRT	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
DXS294	3.26	2.98	2.68	2.06	1.38	0.66
DXS984	1.88	1.74	1.60	1.27	0.90	0.47
DXS1227	2.46	2.28	2.08	1.65	1.17	0.62
DXS292	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
DXS548	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
FRAXAc2	−∞	−1.68	−0.69	0.05	0.25	0.21
DXS1113	−∞	−1.48	−0.71	−0.10	0.10	0.11
G6PD	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
DXS1108	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

*n.i., not informative.

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